Molecular Analysis of Antioxidants (SOD & CAT) in the Sustenance of *Cyamopsis tetragonoloba* (L.) Taub &*Vigna radiata* (L.) Under Fluoride Stress

A Synopsis

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INTRODUCTION:

Plants are often under adverse environmental conditions abiotic factors caused abiotic stress, which play an important role in determining crop productivity (Boyer, 1982) but also the differential distribution of plant species in various types of environment (Chaves *et al.*, 2003) Examples of types of abiotic stress, which may be exposed to a plant, include reduced availability of water, extreme temperatures (heat or freezing), decreased availability of soil nutrients and or excess toxic ions, excess light And higher hardness of the dried soil (Versulues *et al.*, 2006) The ability of plants to adapt to different environments and / or acclimatization is directly or indirectly related to the plasticity and resilience of photosynthesis in combination with other processes that determine the growth and development of the plant, namely, Reproduction (Chaves *et al.*, 2011) A remarkable feature of the adaptation of plants to abiotic stress is the activation of multiple reactions, the Gene interaction complex and crosstalk with many molecular pathways include (Basu, 2012; Umezawa *et al., 2006*) Abiotic stresses evoke complex cellular reactions that have been clarified by advances in research and understanding of plant defenses throughout the plant, physiological, biochemical, cellular and molecular levels (Grover *et al.*, 2001).

Oxidative stress occurs when the production of free radicals and active intermediates in a system's system capacity to neutralize them and to eliminate excess**.** (Sies, 1985; Sies, and Cadenas, 1985)The current concept of "oxidative stress" should also include routes associated with "nitrosative stress" in cellular and extracellular metabolic events for "metabolic stress." Reactive intermediate oxygen (ROI) and intermediate reactive nitrogen (RNI) are continuously produced under physiological conditions (Nathan, 2003; Kröncke, 2003).

The term oxidative stress; It is a state of imbalance in the oxidation of tissues refers to a condition in the when cells were exposed to excessive amounts of molecular oxygen or its chemical derivatives Common Reactive Oxygen Species (ROS). Under physiological conditions, molecular oxygen Subject to a series of reactions that ultimately result in the formation of superoxide anions(O_2-),hydrogen peroxide (H_2O_2) and H_2O.peroxynitrite (OONO), hypochlorous acid (HOCl), radicals (OH), reactive aldehydes.

ROS are produced by normal cellular metabolism and their production is controlled by different enzymatic and non-enzymatic antioxidant systems. Enzymatic antioxidants include CAT, APX, guaiacol peroxidase (GPX), SOD enzymes and LP products and non-enzymatic

detoxifying antioxidants include ascorbic acid (AA), glutathione (GSH), tocopherols (TOC), CARs and phenolic compounds. On the other hand, for the regeneration of the active forms of antioxidants a series of enzymes such MDHAR, reductase dehydroascorbate (DHAR) and GR uses (Apel and Hirt, 2004; Munne-Bosch and Alegre 2004; Zhang et al., 2008). CAT (H_2O_2 oxidoreductase) is a heme-containing enzyme that catalyzes the dismutation of H_2O_2 in H_2O and O_2.

The enzyme is produced in all aerobic eukaryotes, and their function is to eliminate the H_2O_2 produced in peroxisomes of oxidases, which are involved in β-oxidation of fatty acids, photorespiration, catabolism of purines and the oxidative stress (Mittler 2002; Vellosillo et al., 2010). The family of metalloenzymes SOD catalyzes the disproportion of O_2 as H_2O_2 and O_2 and in all aerobic organisms and subcellular components that are sensitive to oxidative stress (del Rio et al., 1996; Halliwell and Gutteridge, 2000; Moussa and Abdel-Aziz, 2008; Chen et al., 2010). While CAT levels of H_2O_2 decreased in peroxisomes, APX fulfills this function in the chloroplasts and cytosol of plant cells.

APX also has its role in H_2O_2 eliminator of two cytosolic forms with defensive paper and a membrane-bound form that modulates quantum efficiency and electron transport in relation to the ascorbate-glutathione (ASA-GSH) (Foyer and Noctor, 2005).GPX is a protein containing heme, monomers of approximately 40 to 50 kDa, it oxidizes certain substrates at the expense of H_2O_2 and releases the cell from excess peroxide, which is produced by metabolism under both normal and stress conditions. GPX decomposed indole-3-acetic acid (IAA) and has a role in lignin biosynthesis and defense against biotic stress due to the consumption of H_2O_2 in the cytosol, vacuole and cell wall and in the extracellular space.GPXs, preferably aromatic electron donors, such as guaiacol and Pyragallol that normally oxidize ascorbate, at a rate of about 1% of that of guaiacol (Asada, 2000; Jebara et al., 2005). (Ashraf *et al.*,2005). Guar (*Cyamopsis tetragonoloba* L.) is one of the future spring-summer legume crops. Guar is mainly grown in the arid and semi-arid areas of Pakistan, India, the United States and South Africa.

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] Cereals are a source of guar gum, which uses many uses in food, food production, textile printing and the pharmaceutical industry. However, the best known use as a reduction of additive friction in drilling mud with crude oil production. Some of the soil microorganisms lives in rhizosphärischen soil plants are known to relieve the Contamination. One of the possible mechanisms to increase drought tolerance in plants by the symbiotic association of environmentally and potentially cost-effective

microbial biofertilizer, ie arbuscular mycorrhizae (AM) -Mongs. Twenty-eight percent of studies on the effects of MA fungi on drought stress in plants have been shown to increase plant growth and power by altering the water relationships of plants, which is the result of Cumulative physical, nutritional and cellular effects. (Auge, 2001). Legumes is important components of agricultural systems in developing countries, both economically and nutritionally. *Cyamopsis Tetragonoloba* (L.) Taub. Commonly known as "guar" is a possible vegetables and cereals industrial leguminöses, which occupies an important part of the weak areas of moisture. As a rich source of chewing gum and protein, it has become an important industrial crop in recent years. Guar gum is a polysaccharide known to have cholesterol-lowering effects. (Butt *et al.,* 2007

Mung bean *Vigna radiata* (L).an important traditional Indian crop is characterized by relatively high protein content and is a short summer harvest season. In addition, mung bean may be an export plant, but soil salinity is a major problem for Leguminosaeproduktion (Abd-Alla *et al*., 1998). Abiotic stresses affecting plant metabolism, destroys cellular homeostasis and decouples the principal physiological and biochemical Prozesse (Arora *et al*., 2002; Pessara and Szaboice, 1999).

Mung bean cultivation *(Vigna radiata* (L) Wilczek) is one of the new crops in Egypt, it is important legumes that are characterized by relatively high protein content and harvest of the short summer season, can also be used as food in a similar way to Faba beans and lentils. In addition, mung bean can be used as a crop with export potential. (Azab, 1997).

Mung bean is very sensitive to salinity stress, so shoot mainly affects root formation in comparison. A very small amount of salt water can cause high reaction in mung bean. (Saha et al., 2010).

Legumes are important sources of food, in addition to the negative effects antinutritioneller factors (tannins, phytates, trypsin inhibitors and hemagglutinins), which limits the use. Germination is the most important way to reduce these factors. This work was designed to explore physiological parameters, the role of antioxidants, biochemical activities, the effect on proteins and other factors in different varieties at different concentrations of fluoride. This is useful for ascertaining the tolerant cultivar under stress fluoride. For the analysis of gene expression in non-leguminöser and leguminöser culture. (Onder and Kahram, 2009**).** This study has been designed to explore the role of antioxidant enzymes in every aspect from

biochemical activity to gene expression analysis in both leguminous crops *Cyamopsis Tetragonoloba* (L.) Taub. and *Vigna radiata* (L) Wilczek.

Review of literature:

Salinity is one of the types of abiotic stress, plant growth and yield productivity (Flowers, 2004). This stress affects seed germination, lack of water, ionic imbalance leading to ions toxicity and osmotic stress (Khan and Panda 2002, Panda and Khan 2003). As a result of ion imbalance and osmotic stress, the primary effect of salt stress is, secondary loads such as oxidative stress occur. The binding of CO2 is limited due to ion imbalance and the osmotic stress condition. It reduces the reduction of carbon in the Calvin cycle and the decrease of oxidized NADP + to serve as an electron acceptor in photosynthesis. It causes ferredoxin to be reduced and electron can pass to oxygen through improved leaks to the formation of superoxide Redikalien (O_2 •) by Mehler reaction. It also generates reactive oxygen species more aggressive, such as hydrogen peroxide (H_2O_2), superoxide (O_2 -) and hydroxyl (OH), which can destroy normal metabolism by oxidative damage to lipids, proteins and nucleic acids (Panda and Khan, 2009).

Since the lower fluoride is absorbed by the roots and then transported through xylematische flow to the Transpirationsorganen. It is mainly transferred to leaves (Davison and Weinstein, 1998). The accumulation of inorganic fluoride by terrestrial plants can be accomplished by deposition of air and ground receptacles (Davison, 1983). In order to alleviate the damages caused by ROS, the plants develop an antioxidant defense system. This defense system includes non-enzymatic molecules, such as proline, carotenoids, ascorbate, glutathione and enzymatic molecules such as SOD, CAT, APX, GR and POD (Salin et al., 1988, Guet-Dahan et al., 1997). All this, enzymatic and non-enzymatic molecules mitigate ROS through their particular acts (Arora et al., 2002). In Arabidopsis thaliana the four members are transcriptionally active with substantially overlapping expression patterns (Emborg et al., 2006).

Jha *et al,* (2009) studied the pot experiment was carried out under controlled conditions to investigate the collection, recording and toxicity of onion fluorine *(Allium cepa L.),* which grew in soil contaminated with inorganic fluoride (NAF). Six different levels of soil contamination were used for 0, 100, 200, 400, 600 and 800 mg of NaF were added to the soil. The results of this study showed that it accumulates more in the unit in the edible part of the onion F as in the onion. The F-accumulation pattern in onion tissues in this study suggests that there is a constant monitoring of onion recording and should not be grown in contaminated F areas.

Bhargava and Bhardwaj, (2010) study the fluoride Contamination in water, soil and plants was an ongoing problem in the world. The effects of 4, 8, 12, 16 and 20 mg / l sodium fluoride (NaF) were in *Triticum aestivum* var. Raj. 4083 seeds and seedling growth. After 7 days of treatment with the control, 100% germination occurred but 20 mg of NaF / L germination was reduced to 88%. The physiological parameters, ie, root length, excess length and dry weight decreased with increasing NaF concentration. At 20 mg NaF / L, mean root length, excess length and dry weight of 36.6%, 24% and 20.54% were reduced. A 20 mg NaF / L Vigor Index on the control was reduced by 37.20%. The chlorophyll content of the leaves was also reduced monotonously. At 20 mg NaF / l was 0.074 mg / g, which was 27.45% lower than the control. The ascorbic acid content initially decreased with increasing NaF concentration (20 mg / L) and then increased with increasing NaF concentration (20 mg / L). Our study concludes that sodium fluoride has a significant influence on the germination and growth of wheat germs.

Saxena and Saxena, (2013) This study provides an overview of the contamination status of groundwater fluoride and shows that there is in Bassi Tehsil acute fluoride problem, since only 36% of groundwater samples have a fluoride content (> 1.5 mg / l, oMS) and the remaining 64% of villages with very high concentrations of fluoride. The favorable factor contributing to the increase in groundwater fluoride is the presence of a rock salt system rich in fluoride. The results of the present study also show that in order to avoid an immediate demand for Defluoridierung techniques and public awareness programs to the population of fluorosis.

Mali *et al,* (2014) the study fluoride can be found in soil, water, air, and even in plants with varying concentrations. This leads to the toxicity of animals and plants. Fluoride was a potent metabolic inhibitor. Therefore, the influence of fluoride concentration (5 ppm, 10 ppm, 25 ppm, 50 ppm and 100 ppm) is the test of photosynthetic pigments (chlorophylls and carotenoids). Total chlorophylls and carotenoids were found to be increased in response to exposure to fluoride. This shows that the *Simarouba glauca* plant stress fluoride by improving photosynthetic efficiency.

Agarwal and Chauhan, (2015) the objective of the present study was to investigate the effects of different concentrations of sodium fluoride in different yields and their bioaccumulation *(Hordeum vulgare)* in barley. Bioaccumulation of fluoride in plant parts demonstrated in plants treated with 18 ppm of NaF in the pot experiment, maximum leaf

accumulation (9.948 mg / kg) and at least in grains (6.302 mg / kg). Field observation of sodium fluoride in the parts of the crop resulted in a maximum accumulation (16.758 mg / kg) in the leaves and a minimum grain (9.351 mg / kg) in the plants thus treated, water tubes in the countryside. The results showed that the use of groundwater, which contains high fluoride content for irrigating barley plants, can tolerate its biological accumulation in several parts of plants.

Ponnusamy and Kumar, (2016) determined the variations in the fluoride content of salt water, salt and the bottom of the pot made nine different Salinas in the belt of the east coast of Tamil Nadu have been documented. Fluoride that is not degradable and is stopped in the zone was estimated by zirconilo alizarin method. Fluoride levels in the Marikina salt (well), were found to be slightly higher than other salt marshes. The chemical parameters used to study the amount of fluoride in saline water masses contain chloride, sulfate, sodium, magnesium, potassium and calcium.

Objectives:

- To Screen of available varieties of *Cyamopsis tetragonoloba* (L.)Taub. and *Vigna radiata* (L.) for tolerance toward fluoride stress on the basis of morphological characteristics.
- Analysis of different biochemical parameters viz photosynthetic pigments, antioxidant activities under concentrations of sodium fluoride along with anti-nutritional factors and inorganic elements of selected variety under fluoride stress.
- To analyze Transcriptional level of the antioxidants (SOD and CAT) in selected varieties of *Cyamopsis tetragonoloba* (L.)Taub. and *Vigna radiata* (L.) under fluoride stress.

Methodology:

Cultivation of the crop plants:

The seeds selected varieties *Cyamopsis tetragonoloba* (L.)Taub. and *Vigna radiata* (L.*)* will be surface sterilized with 0.5% sodium hypochlorite for 10 minutes to avoid contamination by fungi and washed thoroughly with distilled water. These seeds were germinated in a Petri dish containing filter paper soaked Distilled water at 30 ° C under dark conditions. After 3 days, the germinated seedlings were uniformly selected and transferred to plastic tubes containing half strength Hoagland nutrient solution. These pots are placed in a thermostatically cultural area that is maintained s-1 and photoperiod of 16 hours at 25 ± 2 ° C, 500μmol-2. The nutrient solution is changed daily and changed every other day.

Fluoride treatment and sample harvesting:

Seven and fifteen days old, the acclimated seedlings of *Cyamopsis tetragonoloba* (L.)Taub.and *Vigna radiata* (L*)* will be used, respectively, for their later experimental work. Fluoride solution nutrient solution at different concentrations will be added *Cyamopsis tetragonoloba* (L.)Taub.and *Vigna radiata* (L*)* respectively. Stress will be given for 5 days. A control with medium-strength nutrient solution Hoagland without fluoride is used.

Estimation of Physiological parameters:

After the completion of the treatment days, two root and shoot plant samples would be and excess length determination were collected. Fresh weight and dry weight were also recorded.

Lipid peroxidation, H_2O_2, chlorophyll and non-enzymatic antioxidant proline determination:

The degree of lipid peroxidation will be determined by the method of De Vos et al, (1989). Lipid peroxidation was evacuated by examination of the malonaldehyde (MDA) content using an extinction coefficient of 155 mM^{-1} CM^{-1}. H_2O_2 content will be samples from both plants using methods of Alexieva et al, (2001) are measured. The amount of hydrogen peroxide is calculated using a standard curve generated at known concentrations of H_2O_2.

To estimate chlorophyll in fresh leaves *Cyamopsis tetragonoloba* (L.) Taub. and *Vigna radiata* (L.) seedlings will be collected and for the determination of pigments according to the processed Arnon (1949).

The proline content will be calculated in two plants by the method of Bates et al., (1973) it was determined through the standard proline curve.

Estimation Biochemical assay for checking activity of antioxidant enzymes:

- **Extraction of antioxidant enzymes:**
- For the preparation of extracts for the determination of SOD, CAT, and APX, GR activities, the tissue will be homogenized under ice cold conditions in 3ml extraction buffer. This mixture will be centrifuged; the resulting supernatant is stored at -20 ° C for testing various antioxidant enzymes.
- **Enzyme assays and protein determination:**
- The activity will be SOD is assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium by the method of Beauchamp and Fridovich, (1971). CAT activity will be measured by the method of Aebi, (1974).

- The APX activity is the ascorbic acid-dependent H_2O_2 oxidation rate. Its activity will be determined in a reaction mixture, the extract of enzyme phosphate buffer and ascorbic acid would contain (Chen and Asada, 1989).

- GR will be prepared by the method of Smith et al., (1988). This method is based on increasing the absorbance at 412 nm, when 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced by GSH.

- The POD will be test is performed according to the Putter method (1974). Activity POD will be determined by controlling the formation of Tetraguaiacol at 436 nm during the enzymatic reaction of the initial substrate guaiacol in the presence of H_2O_2.

- The protein content of *Cyamopsis tetragonoloba* (L.) Taub and *Vigna radiata* (L.) will be seedlings by the method of Lowry et al., (1951).

Transcriptional level analysis of SOD and CAT of *Cyamopsis tetragonoloba* (L.)Taub and *Vigna radiata* (L.)

We will be performing semi-quantitative RT-PCR to verify the level of SOD and CAT transcription in different parts of *Cyamopsis tetragonoloba* (L.) Taub and *Vigna radiata* (L.), which shows part of maximum response.

First, total we will be RNA isolated from 500 mg samples by grinding with a mortar and pestle in liquid nitrogen using the Trizol reagent and then treated with DNase I free RNase (Promega). Next, the isolated RNA will be reverse transcribed into cDNA in a 20 µl 1 reaction using random hexamers and Superscript II reactions. RT-PCR will be performed with different parts of *Cyamopsis tetragonoloba* (L.) Taub and *Vigna radiata* (L.). To standardize the results, the relative actin will be frequency is determined and used as an internal standard. The number of PCR cycles will be established for each gene to obtain visible bands on agarose gels. Thereafter, will be amplified by the use of primer pair gene-specific PCR by reverse transcription material. The annealing temperature will be standardized with these gene-specific primers. Each set of primers will be amplified using an optimized number of PCR cycles, in order to ensure the linearity requirement for semi-quantitative RT-PCR analysis. We will scan 1.2% on stained ethidium bromide gels and analyze the level of SOD and CAT mRNA in different parts of the plants will be order to check the level of expression, and their identity was confirmed by sequencing.

References:

Apel K. and Hirt H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol, 55:373–399.

Asada K. (2000) the water-water cycle as alternative photon and electron sinks. Phil Trans R Soc Lond B Biol Sci, 355:1419–1431.

Ashraf M.Y., Akhtar K., Sarwar G. and Ashraf M. (2005) Role of the rooting system in salt tolerance potential of different guar accessions. Agron. Sust. Develop, 25:243-249.

Auge R.M. (2001) Water relations drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza,* 11:3-42.

Abd-Alla M.H.,Vuong T.D. and Harper J.E.(1998) Genotypic difference in NaCl stressin intact &grafted soybean. Crop Sci, 38:72.

Arora A., Sairam R.K. and Srivastava G.C. (2002) Oxidative stress & antioxidative system in plants. curr sci, 82:1227.

Azab M.A. (1997) Salt tolerance of two varieties of mungbean (*Vigna radiata* L. Wilczek). Zagazig. J. Agri. Res, 24: 11-21.

Alexieva V., Sergiev I., Mapelli S. and Karanov E. (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ, 24: 1337–1344.

Arnon D.I. (1949) Copper enzymes in isolated chloroplasts: polyphenol oxidases in *Beta vulgaris*, Plant Physiol 24: 1-15.

Aebi H. (1974) Catalases. In: Bergmeyer HU (ed) Methods of enzymatic analysis, Verlag Chemie, Weinheim. Academic Press Inc, New York, p 680.

Arora A. Sairam R.K. and Srivastava G.C. (2002) Oxidative stress and antioxidative system in plants. Curr Sci India, 82: 1227-1238.

Agarwal R. and Chauhan S.S. (2015) Bioaccumulation of sodium fluoride toxicity in plant parts at different phases and its impact on crop *Hordeum vulgare* (Barley) Variety RD 2052. International Journal of Multidisciplinary Research and Development, 2(11): 16-21.

Boyer J.S. (1982) Plant Productivity. Environment Science, 218(4571): 443-448.

Basu U. (2012) Identification of Molecular Processes Underlying Abiotic Stress PlantsAdaptation Using "Omics" Technologies in Benkeblia N. (ed.). Sustainable Agricultureand New Technologies. Boca Raton, CRC Press, 149-172.

Butt M.S., Shahzadi N., Sharif M.K. and Nasu M. (2007) Guar gam a mineral therapy for hypercholesterolemia, hyperglycemia and obesity. Crit. Rev. Food Sci. Nutr, 47:389-396.

Bates L.S., Wadern R.P. and Teare I.D. (1973) Rapid estimation of free proline for water stress determination. Plant Soil, 39: 205–207.

Bhargava D. and Bhardwaj N. (2010) effect of sodium fluoride on seed germination and seedling growth of triticum aestivum var. raj. 4083. journal of phytology, 2(4): 41–43

Chaves M.M., Maroco J.P. and Pereira J.S. (2003) Understanding Plant Responses to Droughtfrom Genes to the Whole Plant. Functional Plant Biology, 30:239-264.

Chaves M.M., Costa J.M. and Saibo N.J.M. (2011) Recent Advances in Photosynthesis under Drought and Salinity. In: Turkan I. (Ed.). Advances in Botanical Research San Diego: Elsevier Ltd, 57:50-83.

Chen Q., Zhang M. and Shen S. (2010) Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (*Helianthus tuberosus* L.). Acta Physiol Plant, 33:273–278.

Chen G.X and Asada K. 1989) Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol 30, 987-998.

del Rio L.A., Palma J.M., Sandalio L.M., Corpas F.J., Pastori G.M., Bueno P. and Lopez-Huertas E. (1996) Peroxisomes as a source of superoxide and hydrogen peroxide in stressed plants. Biochem Soc Trans ,24:434–438.

De Vos CHR., Schat H., Vooijs R. and Ernst WHO. (1989) Copper-induced damage to the permeability barrier in roots of *silene cucubalus*. J Plant Physiol, 135: 164-179.
Davison A. and Weinstein L. (1998) the effects of fluorides on plants. Earth Isl. J, 13: 257-264.

Davison A.W. (1983) Uptake, transport and accumulation of soil and airborne fluorides by vegetation In: Fluorides- Effects on vegetation, animals and humans. Eds. J. L. Shupe, H. B. Peterson and N.C. Leone, Paragon Press, 61-82,

Emborg T.J., Walker J.M., Noh B. and Vierstra R.D. (2006) multiple heme oxygenase family members contribute to the biosynthesis of the phytochrome chromophore in Arabidopsis. Plant Physiol, 140: 856–868.
Foyer C.H. and Noctor G. (2005) Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ, 29:1056–107.

Flowers T. J. (2004) Improving crop salt tolerance. J Exp Bot, 55:307–319.
Grover A., Kapoor A., Laksmi O.S., Agarwal S., Sahi C., Katiyar-Agarwal S., Agarwal M. and Dubey H. (2001) Understanding Molecular Alphabets of the Plant Abiotic Stress Responses. Current Science, 80(2): 206-216.

Gueta-DahanY. Yaniv Z. Zilinskas B.A. and Ben-Hayyim G. (1997) Salt and oxidative stress: Similar and specific responses and their relation to salt tolerance in citrus. Planta, 204: 460-469.

Halliwell B, and Gutteridge J.M.C. (2000) Free radicals in biology and medicine. Oxford University Press, Oxford, UK, Fourth Edition. 136-147.
Jebara S., Jebara M., Limam F. and Aouani M.E. (2005) Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (Phaseolus vulgaris) nodules under salt stress. J Plant Physiol, 162:929–936
Jha S.K., Nayak A.K. and Sharma Y.K. (2009) Fluoride toxicity affects in onion (*Allium cepa L.*) grown in contaminated soils. Chemosphere, 76:353–356.
Kröncke K.D. (2003) Nitrosative stress and transcription. Biological Chemistry, 384:1365-1377. doi:10.1515/BC.2003.153.

Khan M.H. Panda S.K. (2008) Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl salinity stress. Acta Physiol. Plant, 30:81–89.

Khan M.H. and Panda S.K. (2002) Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress. Biol Plant, 45: 625–627.

Lipid Peroxidation End-Products as a Key of Oxidative Stress: Effect of Antioxidant on Their Production and Transfer of Free Radicals.

Lowry O.H., Rosenberg N.J., Farr A.L. and Randall R.J. (1951) Protein measurement with folin phenol reagent. J Biol Chem, 193:265–275.

Munne-Bosch S. and Alegre L. (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol, 31:203–216.

Mittler R. (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci, 7:405–410.

Moussa H.R. and Abdel-Aziz S.M.(2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust J Crop Sci, 1:31–36.

Mali V.V., Bhoite A.S. and Gaikwad D.K. (2014) Influence of fluoride stress on photosynthetic pigments of medicinally important plant *Simarouba Glauca* DC. Indian Journal of Advances in Plant Research (IJAPR), 1(16):1-4.

Nathan C. (2003) Specificity of a third kind Reactive oxygen and nitrogen intermediates in cell signalling. Journal of Clinical Investigation, 111:769-778.

Onder M., Kahraman A. (2009) Antinutritional Factors in Food Grain Legumes. Science and Technology (SözlüSunum). International Symposium on Sustainable Development, Haziran Saraybosnap. 9(10): 40-44.

Pessara M. and Szaboice I. (1999) Soil, salinity & sodicityin Handbook of plant & crop stress.

Putter J. 1974. Peroxidase. In: H.U. Bergemeyer (ed) Methods of Enzymatic Analysis. Vol. II. Academic Press, London, 685-690.

Panda S.K. Khan M.H. (2009) Growth, oxidative damage and antioxidant responses in greengram *(Vigna radiata* L.*)* under short- term salinity stress and its recovery. J Agron Crop Sci, 195: 442-454.

Panda S.K. and Khan M.H. (2003) Salt stress influences lipid peroxidation and antioxidants in the leaf of an indica rice *(Oryza sativa* L*).* Physiol Mol Biol Plants, 9: 273–278.

Ponnusamy S. and kumar R.S. (2016) Comparative study of fluoride with chemical parameters of brine solutions, salt and pan soil from salt pans located in the east coastal belts of Tamil Nadu, India, Research Inventy. International Journal of Engineering and Science, 6(8): 12-24.

Sies, H. (1985) Oxidative stress. Academic Press, San Diego, 1-8.

Sies, H., Cadenas, E. (1985) Oxidative stress Dam- age to intact cells and organs. Philosophical Transactions of the Royal Society of Lond. Biological Sciences, 311:617-631. doi:10.1098/rstb.1985.0168.

Saha P., Chatterjee P. and Biswas A.K. (2010) NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense and osmolyte accumulation in mungbean (Vigna radiata L. Wilczek). Indian J. Exp. Biol, 48:593-600.

Smith I.K., Vierhgeller T.L. and Throne C.A. (1988) Assay of glutathione reductase in crude tissue homogenate using 5, 5'-dithiobis (2-nitrobenzoic acid). Anal Biochem, 175: 408-413.

Salin M.L. (1988) Toxic oxygen species and protective systems of the chloroplast. Physiol Plant, 72: 681-689.

Saxena S. and Saxena U. (2013) Study of fluoride contamination status of ground water in Bassi Tehsil of district Jaipur, Rajasthan, India. International journal of environmental sciences, 3(6): 2251-2260.

Umezawa T., Fujita M., Fujita Y., Yamaguchi S.K. and Shinozaki K. (2006) Engineeringdrought tolerance in plants: discovering and tailoring genes to unlock the future. Current Opinion in Plant Biotechnology, 17(2): 113–122.

Versulues P.E., Agarwal M., Agarwal S.K., Zhu J. and Zhu J.K. (2006) Methods and Concepts in Quantifying Resistance to Drought, Salt and Freezing, Abiotic Stresses that Affect Plant Water Status. The Plant Journal, 45(4): 523-539.

Vellosillo T., Vicente J., Kulasekaran S., Hamberg M. and Castresana C. (2010) Emerging complexity in reactive oxygen species production and signaling during the response of plants to pathogens. Plant Physiol, 154:444–448.

Zhang Y., Yang J., Lu S., Cai J. and Guo Z. (2008) Overexpressing *SgNCED1* in tobacco increases ABA level, antioxidant enzyme activities, and stress tolerance. J Plant Growth Regul, 27:151–158.